Supplementary methods

Lymphocyte phenotyping

Twenty-nine patients underwent longitudinal multi-parameter flow cytometric analysis of lymphocyte subset reconstitution. Analysis of 11 adult healthy volunteers was also performed. Cryopreserved peripheral blood mononuclear cells (PBMC) were thawed and labelled with fluorochrome conjugated antibodies to characterize lymphocyte subsets. Analysis was performed by flow cytometry using a BD FACSCanto II or a BD LSRFortessa. Results were analysed with FlowJo software. Dead cells were excluded from the analysis based on their forward- and side-light scatter properties and use of a Zombie Fixable dead cell staining kit (Biolegend). Monocytes were excluded based on expression of CD14. B cells were defined as CD19+ with naïve (CD24intermediate/ CD38intermediate/IgMintermediate/IgDintermediate/CD27-), transitional (CD24 bright/CD38bright/ IgMbright/IgDbright/CD27-), memory (CD24 bright /CD38dim/ IgMintermediate /IgDdim, CD27+) subsets. Regulatory CD4 T cells were defined as CD3+/CD4+/CD25high/CD27+/CD127low/FoxP3+). CD4 T-cell recent thymic emigrants were defined as CD3+ /CD4+ /CD45RA+ /CD27+ /CD62L+ /CD31+. Cell numbers were calculated from percentage values based on an absolute lymphocyte count of the blood sample obtained using an automated leukocyte counter. Regulatory B cells were defined as Interleukin-10 (IL-10)-producing B cells. PBMC (2.5x106) were cultured with irradiated mouse fibroblast L cells for 24 hours at a ratio of 10:1 in RPMI 1640 with 10% fetal calf serum. CD40L-transfected L cells and non-transfected L cells were kindly provided by Dr Chris Pepper, (Cardiff University, UK). Leukocyte activation cocktail (BD Biosciences) containing phorbol myristate acetate, ionomycin and Brefeldin A was added for the last 6 hours of the culture. Cells were harvested, Zombie Fixable NIR dead cell staining performed followed by incubation with anti CD19-APC antibody. Cells were then fixed and permeabilized (eBioscience), FcR blocking reagent (Miltenyi Biotec) was added and cells incubated with anti-IL-10-PerCP Cy5.5 antibody (clone JES3-9D7) prior to analysis by flow cytometry.

Chimerism

Chimerism was assessed in unfractionated PB CD3+ T cells and CD15+ granulocytes. Full donor chimerism was defined as > 95% donor hematopoietic cells. T-cell subsets were isolated using a BD FACSAria II cell sorter (purity >95%). Genomic DNA was extracted using a QIAamp DNA Micro kit (Qiagen). Percentage donor chimerism was determined using the PowerPlex 16 HS (Promega) multiplex short tandem repeat system for DNA typing. Genetic analysis was performed using an Applied Biosystems 3130xl and results interpreted using ChimerMarker (Version 3.0.9). Chimerism was measured on days 28, 56, 100, 180, 365, and at 2 and 3 years.

Sequencing for somatic mutations

BM was screened for somatic mutations typical for myeloid malignancies in 20 of 27 patients aged > 50 years, using a customised 33 gene diagnostic panel (Illumina TruSeq Custom Amplicon workflow).

Supplementary Table

Toxicity	**Cardiac**	**Pulmonary**	**Hepatic**	**Renal**	**Reaction**
Pre-transplant	4(15%)	12(44%)	4(15%)	10(37%)	
Post-transplant	0	4(33%)	0	10(100%)	10(36%), 1 patient grade 4 reaction
Tolerance to FCC regimen in older subpopulation group. Problems pre and post- transplant associated with individual organ dysfunction					

Supplementary Figures

Figure 1A: Peripheral blood chimerism post-transplant for patients aged ≥50 years

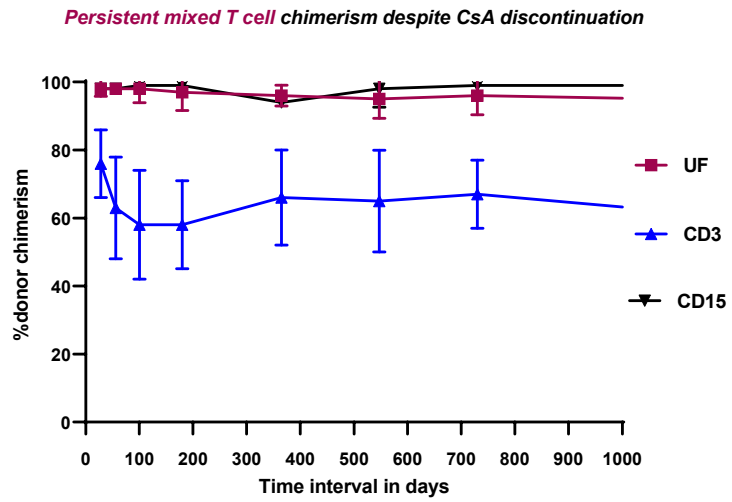

Figure 1B: Peripheral blood chimerism post-transplant for patients aged <50 years

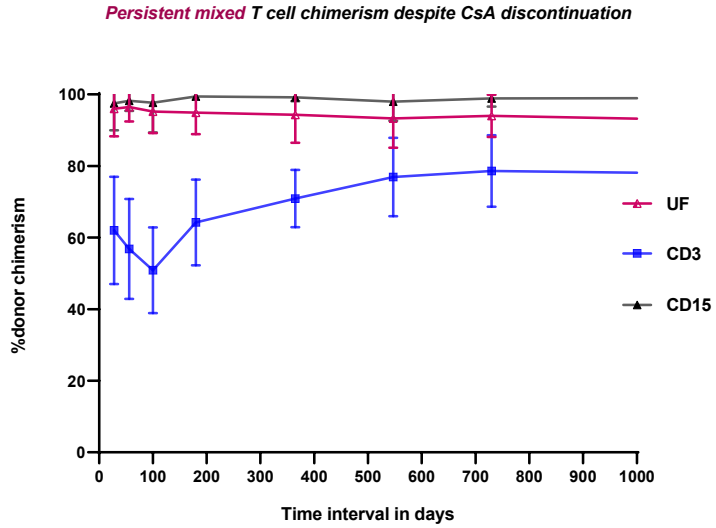

Figure 2: TRM comparison between >50 years and <50 years